

CLAIMS

1. An antibody that binds at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity in NK cells expressing at least one of said two different human inhibitory KIR receptors.
2. The antibody according to claim 1, wherein said antibody is not NKVSF1.
3. The antibody of claim 1, wherein said antibody binds KIR2DL1 and KIR2DL2/3.
4. The antibody of claim 3, wherein said antibody inhibits the binding of a HLA-C allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2/3 receptors.
5. The antibody of claim 4, wherein said antibody binds to substantially the same epitope as monoclonal antibody DF200.
6. The antibody of claim 5, wherein said antibody is a monoclonal antibody or a fragment of a monoclonal antibody.
7. The antibody of claim 6, wherein said antibody is monoclonal antibody DF200 or a fragment thereof.
8. The antibody of claim 1, wherein said antibody is an antibody fragment selected from Fab, Fab', Fab'-SH, F(ab')₂, Fv, diabodies, single-chain antibody fragment, or a multispecific antibody comprising a number of different antibody fragments.
9. The antibody of claim 6, wherein said antibody is a humanized antibody or a chimeric antibody.
10. A hybridoma comprising:

a) a B cell from a non-human mammalian host that has been immunized with an antigen that comprises an epitope present on an inhibitory KIR polypeptide, fused to

b) an immortalized cell,

5 wherein said hybridoma produces a monoclonal antibody that binds at least two different human inhibitory KIR receptor gene products and is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products.

10 11. The hybridoma according to claim 10, wherein said hybridoma does not produce monoclonal antibody NKVSF1.

12. The hybridoma of claim 10, wherein said antibody binds KIR2DL1 and KIR2DL2/3.

15 13. The hybridoma of claim 12, wherein said hybridoma produces an antibody that inhibits the binding of a HLA-c allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2/3 receptors.

20 14. The hybridoma of claim 12, wherein said hybridoma produces an antibody that binds to substantially the same epitope as monoclonal antibody DF200 produced by hybridoma DF200.

15. The hybridoma of claim 14, wherein said hybridoma is DF200.

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16. A method of producing an antibody that binds at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products, said method

30 comprising the steps of:

a) immunizing a non-human mammal with an immunogen comprising an inhibitory KIR polypeptide;

b) preparing antibodies from said immunized animal, wherein said antibodies bind said KIR polypeptide,

c) selecting antibodies of (b) that cross-react with at least two different human inhibitory KIR receptor gene products, and

5 d) selecting antibodies of (c) that capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products, wherein the order of steps (c) and (d) is optionally reversed.

10 17. The method of claim 16, wherein said antibody selected in step c) or d) is not NKVSF1.

18. The method of claim 16, wherein the antibody prepared in step (b) is a monoclonal antibody.

15 19. The method of claim 16, wherein the inhibitory KIR polypeptide used for immunization is a KIR2DL polypeptide and the antibodies selected in step (c) cross-react with at least KIR2DL1 and KIR2DL2/3.

20 20. The method of claim 19, wherein said antibody selected in step (c) inhibits the binding of a HLA-c allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2/3 receptors.

25 21. The method of claim 16, wherein the antibodies selected in step (d) cause an at least about 50% potentiation in NK cytotoxicity.

22. The method according to claim 16, wherein said antibody or antibody fragment binds to substantially the same epitope as monoclonal antibody DF200.

23. The method of claim 18, comprising the additional step of making fragments of the selected monoclonal antibodies.

24. A method of producing an antibody that binds at least two different human inhibitory
5 KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products, said method comprising the steps of:

10 a) selecting, from a library or repertoire, a monoclonal antibody or an antibody fragment that cross-reacts with at least two different human inhibitory KIR2DL receptor gene products, and

b) selecting an antibody of (a) that capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR2DL receptor gene products.

15 25. The method according to claim 24, wherein said antibody selected in step b) is not NKVSF1.

26. The method of claim 24, wherein the antibody selected in step (b) inhibits the
20 binding of a HLA-c allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2/3 receptors.

27. The method of claim 24, wherein the antibody selected in step (b) causes at least a
25 50% potentiation in NK cytotoxicity.

28. The method according to claim 24, wherein said antibody binds to substantially the same epitope as monoclonal antibody DF200.

30 29. The method of claim 24, comprising the additional step of making fragments of the selected monoclonal antibodies.

30. A method of producing an antibody that binds at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products, said method
- 5 comprising the steps of:
- a) culturing a hybridoma of any one of claim 10 to 15 under conditions that cause the expression of said monoclonal antibody; and
 - b) separating said monoclonal antibody from said hybridoma.
- 10 31. The method of claim 30, comprising the additional step of making fragments of the said monoclonal antibody.
32. A method of producing an antibody that binds at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-
- 15 mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products, said method comprising the steps of:
- a) isolating from a hybridoma of any one of claim 10 to 15 DNA encoding said monoclonal antibody;
 - 20 b) optionally modifying said DNA so as to encode for a modified or derivatized antibody selected from a humanized antibody, a chimeric antibody, a single chain antibody or an immunoreactive fragment of an antibody;
 - c) inserting said DNA or modified DNA into an expression vector, wherein said antibody or antibody fragment is capable of being expressed when said
 - 25 expression vector is present in a host grown under appropriate conditions;
 - d) transfecting a host cell with said expression vector, wherein said host cell does not otherwise produce immunoglobulin protein;
 - e) culturing said transfected host cell under conditions which cause the expression of said antibody or antibody fragment; and
 - 30 f) isolating the antibody or antibody fragment produced by said transfected host cell.

33. A pharmaceutical composition comprising an antibody that binds at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on NK cells expressing at least one of said two different human inhibitory KIR receptors, said antibody being present in an amount effective to detectably potentiate NK cell cytotoxicity in a patient or in a biological sample comprising NK cells; and a pharmaceutically acceptable carrier or excipient.
34. The composition of claim 33, further comprising a therapeutic agent selected from an immunomodulatory agent, a hormonal agent, a chemotherapeutic agent, an anti-angiogenic agent, an apoptotic agent, a second antibody that binds to and inhibits an inhibitory KIR receptor, an anti-infective agent, a targeting agent or an adjunct compound.
35. The composition of claim 34, wherein said immunomodulatory agent is selected from IL-1alpha IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-21, TGF-beta, GM-CSF, M-CSF, G-CSF, TNF-alpha, TNF-beta, LAF, TCGF, BCGF, TRF, BAF, BDG, MP, LIF, OSM, TMF, PDGF, IFN-alpha, IFN-beta, or IFN-gamma.
36. The composition of claim 34, wherein said chemotherapeutic agent is selected from alkylating agents, antimetabolites, cytotoxic antibiotics, adriamycin, dactinomycin, mitomycin, carminomycin, daunomycin, doxorubicin, tamoxifen, taxol, taxotere, vincristine, vinblastine, vinorelbine, etoposide (VP-16), 5-fluorouracil (5FU), cytosine arabinoside, cyclophosphamide, thiotepa, methotrexate, camptothecin, actinomycin-D, mitomycin C, cisplatin (CDDP), aminopterin, combretastatin(s), other vinca alkyls, and derivatives or prodrugs thereof.
37. The composition of claim 34, wherein said hormonal agent is selected from leuporelin, goserelin, triptorelin, buserelin, tamoxifen, toremifene, flutamide, nilutamide, cyproterone bicalutamid anastrozole, exemestane, letrozole, fadrozole medroxy, chlormadinone, megestrol, other LHRH agonists, other anti-estrogens, other

anti-androgens, other aromatase inhibitors, and other progestagens.

38. The composition of claim 34, wherein said adjunct compound is selected from phenothiazines, substituted benzamides, antihistamines, butyrophenones, corticosteroids, benzodiazepines, cannabinoids, zoledronic acid, pamidronic acid, erythropoietin, G-CSF, filgrastim, lenograstim, darbepoietin other anti-emetics, other serotonin antagonists, other bisphosphonates or other hematopoietic growth factors.
39. The composition of claim 34, wherein said anti-apoptotic agents is an antisense nucleotide sequence, RNAi, siRNA or small molecule chemical compound that inhibits the expression of a gene selected from bcr-abl, bcl-2, Bcl-x1, Mcl-1, Bak, A1, or A20.
40. The composition of claim 34, wherein said anti-angiogenic agent is selected from neutralizing antibodies, antisense RNA, siRNA, RNAi, RNA aptamers or ribozymes directed against a gene encoding VEGF, a gene encoding a VEGF receptors, VEGF, or a VEGF receptor ; or a variant of VEGF possessing antagonistic properties against VEGF.
41. The composition of claim 34, wherein said second antibody that binds to and inhibits an inhibitory KIR receptor is an antibody or a derivative or fragment thereof that binds to an epitope of an inhibitory KIR receptor that differs from the epitope bound by said antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products.
42. A method of potentiating NK cell activity in a patient in need thereof, comprising the step of administering to said patient a composition of claim 33.
43. The method of claim 42, wherein said patient is suffering from cancer, another proliferative disorder, an infectious disease or an immune disorder.
44. The method of claim 43, wherein said patient is suffering from squamous cell carcinoma, leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell

lymphoma, Burketts lymphoma, acute or chronic myelogenous leukemias, promyelocytic leukemia, fibrosarcoma, rhabdomyosarcoma; melanoma, seminoma, teratocarcinoma, neuroblastoma, glioma, astrocytoma, neuroblastoma, glioma, schwannomas; fibrosarcoma, rhabdomyosarcoma, osteosarcoma, melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer, teratocarcinoma, other carcinoma of the bladder, breast, colon, kidney, liver, lung, ovary, prostate, pancreas, stomach, cervix, thyroid or skin, other hematopoietic tumors of lymphoid lineage, other hematopoietic tumors of myeloid lineage, other tumors of mesenchymal origin, other tumors of the central or peripheral nervous system, or other tumors of mesenchymal origin.

45. The method according to claim 44, wherein said patient is suffering from a hematopoietic tumor of lymphoid lineage.

46. The method according to claim 45, wherein said tumor is selected from T-prolymphocytic leukemia (T-PLL) including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) of the T-cell type; Sezary syndrome (SS); adult T-cell leukemia lymphoma (ATLL); a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma of the pleomorphic or immunoblastic subtype; angio immunoblastic T-cell lymphoma; angiocentric (nasal) T-cell lymphoma; anaplastic (Ki 1+) large cell lymphoma; intestinal T-cell lymphoma; T-lymphoblastic leukemia; or lymphoma/leukemia (T-Lbly/T-ALL).

47. The method of claim 42, wherein said patient is suffering from a proliferative disorder selected from hyperplasias, fibrosis, angiogenesis, psoriasis, atherosclerosis, stenosis or restenosis following angioplasty, and other diseases characterized by smooth muscle proliferation in blood vessels.

48. The method of claim 42, wherein said patient is suffering from an infectious disease caused by a virus selected from hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I (HSV-1), herpes simplex type 2 (HSV-2), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus,

papilloma virus, papilloma virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus or human immunodeficiency virus type I or type 2 (HIV-1, HIV-2).

- 5 49. The method of claim 42, wherein said patient is suffering from an infectious disease caused by a bacteria, protozoa or parasite selected from Staphylococcus, S. pyogenes, Enterococci, Bacillus anthracis, Lactobacillus, Listeria, Corynebacterium diphtheriae, G. vaginalis; Nocardia; Streptomyces; Thermoactinomyces vulgaris; Treponema; Campylobacter, Raeruginosa; Legionella; N.gonorrhoeae; N.meningitides; F. meningosepticum; F. odoratum; Brucella; B. pertussis; B. bronchiseptica; E. coli;
 - 10 Klebsiella; Enterobacter; S. marcescens; S. liquefaciens; Edwardsiella; P. mirabilis; P. vulgaris; Streptobacillus; R. fickettsii; C. psittaci; C. trachomatis; M. tuberculosis, M. intracellulare, M. fortuitum, M. laprae, M. avium, M. bovis, M. africanum, M. kansasii, M. intracellulare; M. lepraemurium; Nocardia, other Streptococcus, other Bacillus, other
 - 15 Gardnerella, other Pseudomonas, other Neisseria, other Flavobacterium, other Bordetella, other Escherichia, other Serratia, other Proteus, other Rickettsiaceae, other Chlamydia, other Mycobacterium, leishmania, kokzidioa, trypanosome, chlamydia or rickettsia.
- 20 50. The method according to claim 42, comprising the additional step of administering to said patient an appropriate additional therapeutic agent selected from an immunomodulatory agent, a hormonal agent, a chemotherapeutic agent, an anti-angiogenic agent, an apoptotic agent, a second antibody that binds to and inhibits an inhibitory KIR receptor, an anti-infective agent, a targeting agent or an adjunct
 - 25 compound wherein said additional therapeutic agent is administered to said patient as a single dosage form together with said antibody, or as separate dosage form.
51. The antibody of claim 1, wherein said antibody is conjugated or covalently bound to a toxin, a detectable moiety, or a solid support.

52. A method of detecting the presence of NK cells bearing an inhibitory KIR on their cell surface in a biological sample or a living organism, said method comprising the steps of:

- 5 a) contacting said biological sample or living organism with an antibody of claim 51, wherein said antibody is conjugated or covalently bound to a detectable moiety; and
- b) detecting the presence of said antibody in said biological sample or living organism.

10 53. A method of purifying from a sample NK cells bearing an inhibitory KIR on their cell surface comprising the steps of:

- a) contacting said sample with an antibody of claim 51 under conditions that allow said NK cells bearing an inhibitory KIR on their cell surface to bind to said antibody, wherein said antibody is conjugated or covalently bound to a solid support;
- 15 and
- b) eluting said bound NK cells from said antibody conjugated or covalently bound to a solid support.

20 54. A composition comprising an antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on NK cells expressing at least one of said two different human inhibitory KIR receptors, wherein said antibody is incorporated into a liposome.

25 55. The composition of claim 54, wherein an additional substance selected from a nucleic acid molecule for the delivery of genes for gene therapy; a nucleic acid molecule for the delivery of antisense RNA, RNAi or siRNA for suppressing a gene in an NK cell; or a toxin or a drug for the targeted killing of NK cells is additionally incorporated into said liposome.

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